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13. ABSTRACT *(Maximum 200 Words)*

The theme and purpose of the grant research was to investigate a number of new ways in which biological processes could be utilized to create new polymeric materials. In the various funded subsections of the grant, there were a number of separate areas of investigation, each studied by a cluster of investigators. The individual areas were: 1) Synthesis and characterization of polymeric materials enzymatically and chemically, incorporating biological macromolecules, in thin film formats to create biosensors and biomaterials; 2) Chemical and enzymatic biopolymer growth and modification; 3) Biopolymer process engineering of N-acylchitosan derivatives; 4) Organic thin films used as templates for bioceramic growth; 5) Enzymatic regioselectivity transesterification of polysaccharides.

14. SUBJECT TERMS

biosensors, photodynamic proteins, enzymatic polytyrosine derivatives, enzymatic transesterification, polysaccharides, chitosan derivatives, Langmuir-Blodgett

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Authors of Report: Dr. Kenneth A. Marx, Dr. Richard Gross, and Dr. Changmo Sung

A. STATEMENT OF THE PROBLEM STUDIED

The theme and purpose of the grant research was to investigate a number of new ways in which biological processes could be utilized to create new polymeric materials. In the various funded subsections of the grant, there were a number of separate areas of investigation, each studied by a cluster of investigators. The individual areas were: 1) Synthesis and characterization of polymeric materials enzymatically and chemically, incorporating biological macromolecules, in thin film formats to create biosensors and biomaterials; 2) Chemical and enzymatic biopolymer growth and modification; 3) Biopolymer process engineering of N-acylchitosan derivatives; 4) Organic thin films used as templates for bioceramic growth; 5) Enzymatic regioselectivity transesterification of polysaccharides.

B. BRIEF SUMMARY FROM ALL GRANT YEARS OF GRANT ACCOMPLISHMENTS IN EACH GRANT SUBSECTION

I. Thin Film Assemblies for Signal Transduction
Professor Kenneth A. Marx, Principal Investigator

Using our 'cassette' methodology, developed for binding biotinylated macromolecules, we created and studied the properties of an optical fiber or glass capillary immobilized alkaline phosphatase biosensor, which was capable of detecting organophosphorus pesticides (down to 500-700 ppb) and Zn (170 ppb), Be (1 ppb) and Bi (2 ppb). In the area of photodynamic proteins, we studied the phycobiliproteins, which we found did not possess strong second order non-linear optical properties. However, they did possess intense two photon (excitation) fluorescence spectra, which persisted in phycobiliproteins entrapped in sol-gel matrices. By contrast, bacteriorhodopsin did possess intense second order non-linear fluorescence spectra and we measured its retained optical properties enhancing the surface plasmon resonance effect, as well as entrapped in sol-gel glasses and mixed with conducting polymers.

We have investigated the enzymatic polymerization, by horseradish peroxidase (HRP), of phenolic and anilinic monomers to form insoluble and soluble conjugated polymers. We demonstrated the free radical polymerization of phenolic monomers in a range of solvents to form controlled MW products in the 1000-3000 size range. These polymers were characterized by a

range of spectroscopic techniques. We took a new approach to immobilizing enzymes by the HRP self-entrapping polymerization into an insoluble matrix. By forming a thin polyphenol film, on an electrode surface, in which HRP was entrapped, we created enzyme electrodes which could electrochemically detect hydrogen peroxide down into the sub-mM range.

In addition, enzyme electrodes were created, either chemically, enzymatically or electrochemically from the combination of bifunctional aromatic diammines. A number of enzymes were entrapped in these matrices that resulted in substrate specific and active systems(Glucose oxidase, HRP, alkaline phosphatase, alcohol oxidase), whose reactions could be monitored electrochemically.

Main chain azopolymers were synthesized from diaminoazobenzene and phenylazobenzene monomers. These polymers exhibited photoisomerization and were characterized spectroscopically.

Water soluble polyphenols and polyanilines were synthesized enzymatically using HRP. As an example, 8-Hydroxyquinoline-5-sulfonate was polymerized using HRP. This polymer was characterized by NMR, including its complexes with Fe(III). Conventional chemical routes for synthesis of water soluble polyanilines require harsh solvents and reagents. Our 'green' conditions are mild by comparison and use no dangerous chemicals or solvents. These polymers were characterized spectroscopically.

HRP was also used to catalyze the aqueous polymerization of monomers of l and d-isomers of amphiphilic C_{10} alkyl tyrosines. In aqueous solutions, these monomers were shown by SEM to be self-assembled into tubules on the micron length scale. Monomers in these tubules could be oligomerized by acting as substrates for HRP reaction, creating tubules with greatly enhanced stability properties. Spectroscopic characterization by FT-IR and NMR was carried out. The kinetics of oligomerization and the pH stability and CMC behavior of these tubule structures was examined by a number of techniques including QCM.

II. Biological Engineering of Polysaccharide Structure

Professor Richard Gross, co-Principal Investigator

The predominant theme of the research funded by this award was how various microbial polysaccharide production systems could be directed towards the formation of 'tailored' polymeric products. *The goal was to achieve levels of control in microbial polysaccharide chain polymerization reactions that are normally associated with traditional chemical routes.*

The focus of our work on polysaccharides has been on controlling molecular weight, polydispersity, the regioselective placement of pendant hydrophobic groups in homopolymers, or the modulation of the ratio of native monomer building blocks. Recently, we have begun to explore the possibility of modulating the main chain composition of polysaccharides. The concept

of directly modulating polysaccharide composition during biosynthesis was prompted by extensive literature reports on the incorporation of non-native monomers into the primary sequence of proteins (modified amino acids) and bacterial polyesters (modified fatty acids). These modifications have resulted in important changes in the functional properties of the corresponding materials (e.g., water absorptivity, thermal transitions, crystallinity, cross-linking behavior). Therefore, we felt a similar approach should be explored for polysaccharides. Surprisingly, we have not found reports in the literature that have used selective-whole cell methods to incorporate modified or non-native sugars into secreted polysaccharides. There is an extensive database on mutation studies to select for altered polysaccharide structures.

To explore this concept, our initial studies focused on curdlan, an unbranched homo-β-(1,3)-glucan produced by *Agrobacterium* sp. The exopolymer was modified *in vivo* by the direct incorporation of the carbon source 3-*O*-methyl-D-glucose (Lee *et al.*, 1997c). Up to 12 mol% of this modified polyglucan was 3-*O*-methyl-D-glucose based on gas chromatography and ^{1}H-NMR analysis. We have not determined whether the 3-*O*-methyl-D-glucose is located in the main chain or as branches. The results of these studies suggested further exploration of this concept.

We chose to focus on cellulose because there are bacterial sources of the polymer and there is extensive literature on growth requirements, biosynthesis, and the formation of fibrils. In addition, useful features of bacterial cellulose include a high degree of crystallinity, excellent mechanical strength, large absorption capacity (fibrils have ~300 fold greater surface area than wood-derived cellulose (Kent *et al.*, 1991)), and high chemical purity. The latter property results in substantially reduced processing requirements in comparison to cellulose derived from plant sources (Ross *et al.*, 1991). The mechanical properties of bacterial cellulose sheets are impressive, with Young's modulus >15 GPa (Yamanaka *et al.*, 1989). The incorporation of the bacterial cellulose into paper results in improved paper strength. Reviews on bacterial cellulose synthesis and its unique characteristics have been published (for examples, see Ross *et al.*, 1991; Salmon and Hudson, 1997).

We have recently completed preliminary studies on the biosynthesis of cellulose and modified cellulose by *A. xylinum.* These studies support the concept that the non-glucose analogs 2-amino-2-deoxy-glucose and 2-acetimido-2-deoxy-glucose can be directly incorporated into microbial cellulose formed by *A. xylinum* (Table 1).

Table 1. Preliminary data to demonstrate the incorporation of non-native sugars into cellulose. Fractionation of exopolymers made with glucose (Glu) analogs (glucosamine = AmGlu, N-acetylglucosamine = AcGlu) fed to *A. xylinum.*

		Insoluble Fraction:		Soluble Fraction:	
Carbon Source:	Fractionation Method	Amount (%)	Molar Ratio (Glu:AmG or Glu:AcG)	Amount (%)	Molar Ratio (Glu:AmG or Glu:AcG)
Glucosamine	Acetic Acid	90	6.0:1.0	10	0.6:1.0
N-Acetylglucosamine	Distilled Water	90	6.2:1.0	10	0.8:1.0

This strategy resulted in the formation of novel copolymers, glucose/2-amino-2-deoxy-glucose and glucose/2-acetimido-2-deoxy-glucose. Our studies suggest that about 15 mol% of the repeat units in the exopolymer produced had the structure of the carbon source used (2-amino-2-deoxy-glucose or 2-acetimido-2-deoxy-glucose). These conclusions were based on extensive purification of the exopolymers followed by GC, GC/MS and NMR analyses. The cellulose-chitin and cellulose-chitosan copolymers have not been previously reported and represent a new group of polysaccharides based on solubility, functional properties and structure. The concept of forming biologically derived copolymers of cellulose provides new options for these materials, both in terms of insights into fundamental metabolic controls of the synthesis and assembly process, as well as the functional properties of these new products. Although polymer blends of cellulose-chitin and cellulose-chitosan have been reported, the availability of copolymers with these monomers is expected to result in novel properties as well as enhanced control over structural features. This has already been observed based on the unique solubility behavior of the new copolymers. In addition, direct formation of fibers by the bacterium will be useful in the study of crystallinity and mechanical properties. It is worth noting that the process to generate novel copolymers (cellulose-chitin, cellulose-chitosan) ameliorates the inherent difficulties associated with modifying plant-derived cellulose. In addition, this direct one-step process offers a simpler and less 'environmentally-damaging' approach (in terms of organic solvents, catalyst) towards new useful degradable polymers.

In addition to the above, we have also studied modulation of the native sugar compositions so that unique copolymers could be formed. For example, we have reached compositional extremes for the component sugars (rhamnose and glucose) of heteropolysaccharide-7 (PS-7) produced by *Beijerinckia indica.* Similarly, highly unusual copolymers were formed that consisted of glucose and galactose in zoogloea gum from *Zoogloea ramigera.*

III. Biopolymer Processing

Professor Steve McCarthy, co-Principal Investigator

This co-Principle Investigator did not provide a written description of this sub-area for this report.

IV. Transmission Electron Microscope (TEM) Investigation

Professor Changmo Sung, co-Principal Investigator

We have carried out experiments in the area of bioceramics. In these experiments, TEM analysis was performed on organic LB films, which served as substrates for the growth of calcium carbonate and other crystals as a function of varying the LB trough subphase conditions. Single crystal electron diffraction analysis of the growth stages was performed. Special attention was paid to analyzing the interface of film cracks and grain boundaries. Nucleation density and size distribution of crystals under different LB monolayers was investigated by the above techniques. SEM analysis, through energy dispersive X-ray spectroscopy, was also performed on a range of alumina and aluminum hydroxide samples. These were both powder samples produced by a variety of solution conditions as well as thin LB film samples in which the particles were grown off the LB monolayer film subphase face.

V. Enzymatic Transesterification of Polysaccharides in Organic Solvents

Dr. Kenneth A. Marx, Principal Investigator

A novel method to achieve enzyme solubilization in organic solvents through formation of hydrophobic ion-pairs between an enzyme and a charged surfactant, such AOT, was invented and used. In particular, we observed that protease N (*Bacillus subtilis*), and protease S (*Bacillus specific*) ion paired with the anionic surfactant aerosol OT, AOT, remained predominantly active in isooctane. Such system was implemented for the modification of polysaccharides in organic solvents using different vinyl esters. Polysaccharides such as amylose, cellulose, cyclodextrins, hydroxy ethyl cellulose, pullullan, and maltodextrin were the substrates for the enzyme. The final product was characterized by different methods. ^{1}H NMR revealed that alcohol 6 was the site for a regioselective transesterification. Such polysaccharides are hydrophobic and less prone to oxygen penetration.

The ion paired protease enzyme was also used for the ring opening polyesterification of different lactones such as ε-caprolactone (ECL), 3S-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione (DILAC), γ-butirrolactone (GBL), and δ-valerolactone (DVL), in reasonable times, for industrial applications. Copolymers using ECL, DILAC, and DVL were synthesized and characterized. These polymers were of great interest for their mechanical properties, for their biodegradability and for their applications in drug delivery.

C. LIST OF MANUSCRIPTS submitted or published under ARO sponsorship during this reporting period, INCLUDING JOURNAL REFERENCES:

I. Thin Film Assemblies for Signal Transduction
Professor Kenneth A. Marx, Principal Investigator

Manuscripts Published:

"Mechanistic Study Of Enzyme Catalyzed Polymerization Of 8-Hydroxyquinoline-5-Sulfonate Using Nuclear Magnetic Resonance Spectroscopy" (K.S. Alva, K.A. Marx, J. Kumar and S.K. Tripathy) *Macromolecular Rapid Communications,* 18 133-137 (1997).

"Two-photo-induced Fluorescence fro the Phycoerythrin Protein" (Z. Chen, D.L. Kaplan, K. Yang, J. Kumar, K.A. Marx and S.K. Tripathy) *Applied Optics,* 36 7 (1997).

"Novel Immobilization Techniques in the Fabrication of Efficient Electrochemical Biosensors" (K.S. Alva, K.A. Marx, L.A. Samuelson, J. Kumar, S.K. Tripathy and D.L. Kaplan) Smart Structures and Materials (A. Crowson, Editor) SPIE 2716 152-163 (1996).

"Enzymatic Polymerization of Amphiphilic Alkyl Tyrosine Derivatives From Emulsions" (R. Sarma, K.S. Alva, K.A. Marx, S.K. Tripathy, J.A. Akkara and D.L. Kaplan) *Materials Science and Engineering* C4 189-192 (1996).

"New Strategies for the Fabrication of Enzyme Electrodes" (K.S. Alva, L.A. Samuelson, J. Kumar, K.A. Marx, D.L. Kaplan and S.K. Tripathy) Proceedings MRS Materials Research Society, Pittsburgh, Pennsylvania 414 119-124 (1996).

"Multilayer Enzyme Assembly for the Development of a Novel Fiber Optic Biosensor" (Z. Chen, H.H. Gao, J. Kumar, K.A. Marx, S.K. Tripathy and D.L. Kaplan) Proceedings MRS Materials Research Society, Pittsburgh, Pennsylvania 414 125-132 (1996).

"Signal Transduction Composites: Biomaterials with Electroactive Polymers" (L.A. Samuelson, R. Pande, M. Ayyagari, S.D. Kamtekar, K.G. Chittibabu, H. Gao, K.A. Marx, J. Kumar, S.K. Tripathy and D.L. Kaplan) Polymeric Materials Encyclopedia: Synthesis, Properties and Applications (J.C. Salamone, Editor) Polymer Science and Technology Series 10, QS 7612-7620 (1996).

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"Biochemical Synthesis Of Water Soluble Polyanilines: Poly(P-Amino Benzoic Acid)" (K.S. Alva, K.A. Marx, J. Kumar and S.K. Tripathy) *Macromolecular Rapid Communications* 17 859-863 (1996).

"Horseradish Peroxidase Catalyzed Polymerization Of Amphiphilic Tyrosine Derivatives In Micelles" (R. Sarma, K.S. Alva, K.A. Marx, S.K. Tripathy, J.A. Akkara and D.L. Kaplan) Proceedings MRS Materials Research Society, Pittsburgh, Pennsylvania (1996).

"Trace Analysis of Zn(II), Be(II) and Bi(III) by Enzyme-Catalyzed Chemiluminescence" (S.D. Kamtekar, R. Pande, M.S. Ayyagari, K.A. Marx, D.L. Kaplan, J. Kumar and S. Tripathy) *Analytical Chemistry* 68 216-220 (1996).

"A Biotinylated Undecylthiophene Copolymer Bioconjugate for Surface Immobilization: Creating an Alkaline Phosphatase Chemiluminescence Based Biosensor" (R. Pande, S.D. Kamtekar, M. Ayyagari, M. Kamath, K.A. Marx, J. Kumar, S.K. Tripathy and D.L. Kaplan) *Bioconjugate Chemistry,* 7 159-164 (1996).

"Sol-gel Encapsulated Light-Transducing Protein Phycoerythrin: A New Biomaterial" (Z. Chen, L.A. Samuelson, J. Akkara, D.L. Kaplan, H. Gao, J. Kumar, K.A. Marx and S.K. Tripathy) *Chemistry of Materials* 7 1779-1783 (1995).

"Enzyme-Mediated Two-Dimensional Polymerization of Aromatic Derivatives on a Langmuir Trough" (F.F. Bruno, J.A. Akkara, D.L. Kaplan, P. Sekher, K.A. Marx and S.K. Tripathy) *Industrial and Engineering Chemistry Research* 34 4009-4015 (1995).

"Chemiluminescence-Based Inhibition Kinetics of Alkaline Phosphatase in the Development of a Pesticide Biosensor" (M.S. Ayyagari, S. Kamtekar, R. Pande, K.A. Marx, J. Kumar, S.K. Tripathy, J. Akkara and D.L. Kaplan) *Biotechnology Progress* 11 699-703 (1995).

"Enzymatic Mediated Synthesis of Conjugated Polymers at the Langmuir Trough Air-Water Interface" (F.F. Bruno, J.A. Akkara, L.A. Samuelson, D.L. Kaplan, B.K. Mandal, K.A. Marx, J. Kumar and S.K. Tripathy) *Langmuir* 11(3) 889-892 (1995).

"Molecular Assembly of Proteins and Conjugated Polymers: Toward Development of Biosensors" (M.S. Ayyagari, R. Pande, S. Kamtekar, H. Gao, K.A. Marx, J. Kumar, S.K. Tripathy, J.A. Akkara and D.L. Kaplan) *Biotechnology and Bioengineering* 45 116-121 (1995).

"Controlled Free Radical Polymerization of Phenol Derivatives by Enzyme-Catalyzed Reactions in Organic Solvents" (M.S. Ayyagari, K.A. Marx, S.K. Tripathy, J.A. Akkara and D.L. Kaplan) *Macromolecules* 28 5192-5197 (1995).

"Biosensors for Pesticide Detection Based on Alkaline Phosphatase-Catalyzed Chemiluminescence" (M.S. Ayyagari, S. Kamtekar, R. Pande, K.A. Marx, J. Kumar, S.K. Tripathy and D.L. Kaplan) *Materials Science and Engineering* C2 191-196 (1995).

"A Chemiluminescence-Based Biosensor for Metal Ion Detection" (S.D. Kamtekar, R. Pande, M.S. Ayyagari, K.A. Marx, D.L. Kaplan, J. Kumar and S. Tripathy) *Materials Science and Engineering* C3 79-83 (1995).

"Tapered Fiber Tips for Fiber Optic Biosensors" (H.H. Gao, Z. Chen, J. Kumar, S.K. Tripathy and D.L. Kaplan) *Optical Engineering* 34(12) 3465-3469 (1995).

"Integrating Biotinylated Polyalkylthiophene Thin Films with Biological Macromolecules: Biosensing Organophosphate Pesticides and Metal Ions with Surface Immobilized Alkaline Phosphate Utilizing Chemiluminescence Measurements" (R. Pande, S. Kamtekar, M. Ayyagari, K.A. Marx, J. Kumar, S.K. Tripathy and D.L. Kaplan) Smart Structures and Materials (A. Peter Jardine, Editor) SPIE 2441 12-22 (1995).

"Synthesis and Characterization of Polyphenols and Polyanilines From Peroxidase Mediated Reactions on a Langmuir Trough" (F.F. Bruno, J.A. Akkara, L.A. Samuelson, D.L. Kaplan, B.K. Mandal, K.A. Marx and S.K. Tripathy) Intelligent Materials and Systems (P. Vincenzini, Editor) Techna, Farnza, Italy 99-106 (1995).

"Advanced Materials From Enzymatic Polymerization of Substituted Phenols in Ordered Templates" (M. Ayyagari, F. Bruno, S.K. Tripathy, K.A. Marx, D.L. Kaplan, J. Akkara and D. Rao) Polymers and Other Advanced Materials: Emerging Technologies and Business Opportunities (P.N. Prasad, J.E. Mark and T.J. Fai, Editors) Plenum Press, New York 667-675 (1995).

Manuscripts to be Published:

"Study Of Chemical Reactions By In-Situ Nuclear Magnetic Spectroscopy" (K.S. Alva, A.L. Cholli, K.A. Marx, J. Kumar and S. Tripathy) *Polymer Preprints, in press* 1998.

"Biochemically Designed Polymers As Self Organized Materials" (K.S. Alva, R. Sarma, K.A. Marx, J. Kumar, S.K. Tripathy, J. A. Akkara and D. L. Kaplan) SPIE Proceedings *in press* 1998.

"Biochemical Synthesis Of Electroactive Polymers" (K.S. Alva, J. Kumar, K.A. Marx and S.K. Tripathy) SPIE Proceedings *in press* 1998.

"Horseradish Peroxidase Catalyzed Polymerization Of Amphiphilic Tyrosine Derivatives In Micelles" (R. Sarma, K.S. Alva, K.A. Marx, J.A. Akkara, D.L. Kaplan and S.K. Tripathy) SPIE Proceedings *in press* 1998.

"Molecular Assembly of Multilayer Enzyme: Towards the Development of Chemiluminescence Based Fiber Biosensors" (Z. Chen, H. Gao, J. Kumar, K.A. Marx, S.K. Tripathy and D.L. Kaplan) *Materials Science & Engineering, in press* 1998.

Manuscripts Submitted for Publication:

"Phycobiliproteins Encapsulated in Sol-Gel Glass" (Z. Chen, D.L. Kaplan, K. Yang, J. Kumar, K.A. Marx and S.K. Tripathy) submitted to *Sol-Gel Science and Technology,* 1998.

"Enzymatic Synthesis And Characterization Of A Novel Water Soluble Polyaniline: Poly(2,5 Diaminobenzene Sulfonate" (K.S. Alva, J. Kumar, K.A. Marx and S.K. Tripathy) submitted to *Macromolecules* 1998.

"Bacteriorhodopsin Encapsulated in Conducting Polymer: A Biomaterial for Signal Transduction" (Z. Chen, K.G. Chittibabu, L. Li, K.A. Marx, J. Kumar, S.K. Tripathy and D.L. Kaplan) submitted to *Advanced Materials* 1998.

II. Biological Engineering of Polysaccharide Structure
Professor Richard Gross, co-Principal Investigator

"Exopolymers From Curdlan Production-incorporation of Glucose-Related Sugars by *Agrobacterium sp.* ATCC 31749" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan, F. Deng and R.A. Gross) *Canadian Journal of Microbiology* 43 149-156 (1997).

"Compositional Consistency of a Heteropolysaccharide-7 Produced by *Beijerinckia indica*" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan and R.A. Gross) *Biotechnology Letters* 19(8) 803-807 (1997).

"Production of Zoogloea gum by Zoogloea ramigera with Glucose Analogs" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan and R.A. Gross) *Biotechnology Letters* 19(8) 799-802 (1997).

"Microbial Production of Water-soluble Non-curdlan Type Exopolymer-B with Controlled Composition by Agrobacterium sp. ATCC 31749" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan and R.A. Gross) *Biotechnology Letters* 19(12) 1217-1221 (1997).

"Exopolymers From Curdlan Production-incorporation of Glucose-related Sugars by Alcaligenes faecalis var. myxogenes ATCC 31749" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan, F. Deng and R.A. Gross) The 96th General Meeting of American Society of Microbiology (ASM) New Orleans, LA, May 19-23, 1996.

"Biosynthesis of Novel Exopolymers by Aureobasidium pullulans" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan, F. Deng and R.A. Gross) *manuscript in preparation* 1998.

"Formation of Glucosamine-glucose Copolymers by a Cellulose Producing Strain" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan, F. Deng and R.A. Gross) *manuscript in preparation* 1998.

IV. Transmission Electron Microscope (TEM) Investigation
Professor Changmo Sung, co-Principal Investigator

"Microstructures of Synthetic Aragonite Grown under Organic Template" (C.S. Sung) *Journal of Materials Science, in press* 1997.

"TEM/XRD Analysis of Ba-Sr-Ti Oxide Crystallization at an Air-Liquid Interface" (C.S. Sung) submitted to *Journal of Materials Research* 1997.

V. Enzymatic Transesterification of Polysaccharides in Organic Solvents
Dr. Kenneth A. Marx, Principal Investigator

"Regioselective Enzymatic Transesterification of Polysaccharides in Organic Solvents" (F.F. Bruno, J.A. Akkara, D.L. Kaplan, R. Gross, J.S. Dordick and G. Swift) in Enzymes in Polymer Synthesis ACS Symposium Series 167 (1998).

"Regioselective Enzymatic Transesterification of Polysaccharides in Organic Solvents" (F.F. Bruno, J.S. Dordick, D.L. Kaplan and J.A. Akkara) *Macromolecules, in press* 1998.

"Enzyme Catalysis for Polymer Synthesis and Modification" (M. Ayyagary, F.F. Bruno and J.A. Akkara) *Biotechnology, in press* 1998.

"Enzymatic Modification of Insoluble Amylose in Organic Solvents" (F.F. Bruno, J.A. Akkara, D.L. Kaplan, R. Gross, J.S. Dordick and G. Swift) *Macromolecules* 28 8881 (1995).

D. SCIENTIFIC PERSONNEL supported by this project and HONORS/AWARDS/DEGREES received during this reporting period:

I. Thin Film Assemblies for Signal Transduction
Professor Kenneth A. Marx, Principal Investigator

Dr. Kenneth Marx	Dr. Jayant Kumar	Dr. Sukant Tripathy
Dr. Tiean Zhou	Dr. Madhu S.R. Ayyagari	Dr. Zhongping Chen
	Dr. Shridhara Alva	

Ph.D. Degrees Awarded

Dr. Rupmoni Sarma

Dr. Harry Gao

Dr. Rajiv Pande

Dr. Sanjay Kamtekar

II. ***Biological Engineering of Polysaccharide Structure***
Professor Richard Gross, co-Principal Investigator

Dr. Richard Gross Dr. Jin Woo Lee Mr. Fang Deng

IV. ***Transmission Electron Microscope (TEM) Investigation***
Professor Changmo Sung, co-Principal Investigator

Dr. Changmo Sung

V. ***Enzymatic Transesterification of Polysaccharides in Organic Solvents***
Dr. Kenneth A. Marx, Principal Investigator

Dr. Ferdinando Bruno Dr. Tiean Zhou

E. Report of INVENTIONS (By TITLE ONLY):

I. ***Thin Film Assemblies for Signal Transduction***
Professor Kenneth A. Marx, Principal Investigator

"A Chemiluminescence-Based Method for Rapid and Sensitive Detection of Organophosphorus Compounds" (M. Ayyagari, K. Marx, J. Akkara, D.L. Kaplan, S. Kamtekar, R. Pande, S.K. Tripathy, J. Kumar) Patent Pending.

"Electrochemical Biosensor Arrays" (L.A. Samuelson, K.S. Alva, D.L. Kaplan, K.A. Marx, S. Tripathy, J. Kumar, J. Akkara, P. Dunne, G. Wallace and K. McGrath) Patent Pending.

II. ***Biological Engineering of Polysaccharide Structure***
Professor Richard Gross, co-Principal Investigator

"Process to Produce Modified Polysaccharide by Direct Incorporation of Modified Sugars" (J.W. Lee, W.G. Yeomans, A.L. Allen, D.L. Kaplan, F. Deng and R.A. Gross) Patent Application Filed.

IV. ***Transmission Electron Microscope (TEM) Investigation***
Professor Changmo Sung, co-Principal Investigator

No patent disclosures to date.

V. ***Enzymatic Transesterification of Polysaccharides in Organic Solvents***
Dr. Kenneth A. Marx, Principal Investigator

"Enzymatic Transesterification of Insoluble Polysaccharides in Organic Solvents" (F.F. Bruno, J.A. Akkara, D.L. Kaplan and J.S. Dordick) Patent Pending.